

### ABSTRACT

A method is provided for rapidly and reliably constructing recombinant herpes simplex virus (HSV) capable of expressing a target protein in cancer cells. The method for constructing recombinant HSV as claimed in the present invention

5 comprises a first step of inserting into a herpes simplex virus (HSV) genome, a BAC plasmid, which has a loxP site and an FRT site and into which has been inserted at least one type of marker gene expression cassette between the loxP site and the FRP site, a second step of constructing a shuttle vector into which has been

10 respectively inserted at least one type of expression cassette of a gene encoding a target protein, at least one type of marker gene, a loxP site and an FRP site, and inserting the shuttle vector into the loxP site of the HSV genome using Cre recombinase, and a third step of co-infecting a host with the HSV genome and a vector capable of expressing Flp recombinase, and excising the region between the FRT sites in the genome to produce a target recombinant HSV.